American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:121–128 (1997)

Linkage Studies Suggest a Possible Locus for Bipolar Disorder Near the Velo-Cardio-Facial Syndrome Region on Chromosome 22

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Velo-cardio-facial syndrome (VCFS) is a congenital anomaly characterized by multiple dysmorphisms, cleft palate, cardiac anomalies, and learning disabilities, that results from a microdeletion of chromosome 22q11. An increased prevalence of psychiatric illness has been observed, with both schizophrenia and bipolar disorder commonly being diagnosed. For these reasons, the VCFS region is an interesting candidate region for bipolar disorder. We examined this region in 17 bipolar families from three populations: 13 families from the general North American population (University of California, San Diego/University of British Columbia, UCSD/UBC), three larger families from New York, and a portion of Old Order Amish pedigree 110. Three microsatellite markers spanning 13 cM around the VCFS region were genotyped in all the families. A maximum lod score of 2.51 was obtained in the UCSD/UBC families under a dominant model at D22S303. In the combined family set, maximum lod scores of 1.68 and 1.28 were obtained at this marker under dominant and recessive models, respectively. Four additional markers were subsequently typed in selected positive families, and yielded positive lods at 6 of 7 markers spanning 18 cM in this region. Nonparametric, multipoint analyses using the affected pedigree member (APM) method also yielded suggestive evidence for linkage in both the UCSD/UBC family set ($P = 0.0024$) and in the combined families ($P = 0.017$). Affected sib-pair analyses were similarly positive in the UCSD/UBC families ($P = 0.017$), and in the combined families ($P = 0.004$). These results are suggestive of a possible locus for bipolar disorder near the VCFS region on chromosome 22. Am. J. Med. Genet. 74:121–128, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: bipolar disorder; genetic linkage; velo-cardio-facial syndrome; chromosome 22; VCFS; manic depressive illness

INTRODUCTION

Bipolar disorder (manic depressive illness) is a common psychiatric illness characterized by recurrent episodes of mania and depression. Twin, family, and adoption studies show that the vulnerability to develop bipolar disorder is inherited [Nurnberger et al., 1994; Bertelsen et al., 1977; Nurnberger and Gershon, 1984]. However, despite years of investigation, the exact mode of inheritance is not well-understood. Although some family studies have supported the existence of autosomal-dominant major loci, a simple Mendelian pattern of inheritance is usually not observed [Spence et al., 1995; Rice et al., 1987; Sham et al., 1992; Faraone et al., 1990]. The mode of inheritance in bipolar disorder is an important concern since linkage analysis, which is used to identify the chromosomal position of disease-causing alleles as a prelude to positional cloning, is most successfully exploited for single-gene defects. Evi-

Contract grant sponsor: NIMH; Contract grant number MH47612; Contract grant sponsor: UCSD Mental Health Clinical Research Center; Contract grant number MH30914; Contract grant sponsor: UCSD General Clinical Research Center; Contract grant number MO1 RR00827.

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Received 2 June 1995; Revised 19 September 1996

dence for linkage to the short arm of chromosome 11 in an Old Order Amish bipolar pedigree and the X-chromosome in several Israeli families was provided in some early linkage studies [Egeland et al., 1987; Baron et al., 1987]. However, these findings have not been replicated, and the strong support for chromosome 11 linkage was substantially diminished following further analysis of the original pedigrees [Kelsoe et al., 1989; Berrettini et al., 1990; Gershon, 1991; Baron et al., 1993]. A recent genome-wide search also failed to provide convincing evidence for linkage to any chromosomal locus [Coon et al., 1993]. Recently, using nonparametric methods of statistical analysis [Weeks and Lange, 1988; Blackwelder and Elston, 1985; Risch, 1990] in conjunction with conventional lod score analysis, evidence was obtained for linkage to chromosomes 18 and 21 in subsets of bipolar families [Berrettini et al., 1994; Straub et al., 1994; Gurling et al., 1995; Stine et al., 1995; Freimer et al., 1996]. More recently, evidence for linkage has been reported on 6p24, 13q13, 15q11, and 4p16 [Ginns et al., 1996; Blackwood et al., 1996]. However, these findings await replication.

In the search for genes for complex illnesses such as bipolar disorder, an alternative to a labor-intensive whole-genome survey is to analyze specific candidate genes or loci. Although it is difficult to target appropriate candidate genes in bipolar disorder, a number of dopaminergic and adrenergic receptor gene loci have been investigated. So far, no evidence for linkage has been reported for these candidate genes [Holmes et al., 1991; Mitchell et al., 1992; De Bruyn et al., 1994]. Candidate loci in bipolar disorder could also be targeted if one could identify clinical entities that are caused by well-characterized chromosomal defects that are also associated with psychiatric illness. For this reason, we investigated chromosome 22q11, which is involved in velo-cardio-facial syndrome (VCFS).

VCFS is a phenotypically heterogeneous congenital anomaly, that is usually characterized by cleft palate, typical facial appearance, cardiac abnormalities, and learning disability [Shprintzen et al., 1978]. In addition, approximately two dozen minor clinical features have been described including scoliosis, renal aplasia, T-cell deficiency, and hypocalcemia. Fluorescent in situ hybridization (FISH) and molecular analysis indicate that VCFS is caused by microdeletions on chromosome 22q11, with a defined minimally deleted region of approximately 1–3 megabases [Scambler et al., 1992; Lindsay et al., 1993; Morrow et al., 1995].

A number of behavioral disorders have been identified in patients with VCFS during early childhood, including separation anxiety disorder, attention-deficit disorder, school phobia, obsessive-compulsive disorder, mood disorders, and paranoid disorder [Shprintzen et al., 1978; Chow et al., 1994; Goldberg et al., 1993]. As these patients reached adolescence, approximately 30% of them developed psychotic symptoms [Shprintzen et al., 1992]. In a recent study of 14 patients with VCFS, 4 were given a diagnosis of either schizophrenia or schizoaffective disorder [Pulver et al., 1994c]. An additional 7 patients were found to have alcohol abuse, obsessive-compulsive disorder, social phobia, or adjustment disorder with depressed mood. In addition, in a study of 100 patients with schizophrenia, 2 were found to have 22q11 microdeletions consistent with VCFS [Lindsay et al., 1995]. However, an ongoing evaluation of adolescents with VCFS suggests a pattern of symptoms that falls more within the bipolar disorder spectrum [Papolos et al., 1996].

Since deletion of a gene on chromosome 22q11 appears to be a factor in the development of severe psychiatric illness in VCFS patients, some of whom appear to have bipolar disorder, we wanted to determine whether this region could also be responsible for bipolar vulnerability in the general population. We therefore used highly polymorphic dinucleotide repeat markers that flank the VCFS region on chromosome 22q11 to determine whether linkage could be established in 17 multiplex families with bipolar disorder. We here report linkage evidence suggestive of a locus for bipolar disorder in this region.

MATERIALS AND METHODS

Family Ascertainment and Diagnosis

Families for this study were ascertained from four sites: San Diego, Vancouver, New York, and Pennsylvania. The size and clinical characteristics of these families are shown in Table I. Thirteen of these families derive from an ongoing collaboration between the University of California, San Diego (UCSD) and the University of British Columbia (UBC). These families represent a collection of small-to-medium size families from the general population selected for a bipolar proband, and at least two other members with bipolar or recurrent unipolar affective disorder. Families in San Diego were selected from UCSD hospitals and clinics, and from the UCSD Mental Health Clinical Research Center Outpatient Clinic. Families in Vancouver were ascertained from the UBC Mood Disorders Clinic [Sadovnick et al., 1994]. Family members at these two sites were interviewed using the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer et al., 1987] by clinician interviewers who had undergone the same

TABLE I. Characteristics of Bipolar Families

	UCSD/UBC	New York	Amish
Number of families	13	3	1
Total subjects	123	31	38
Average members per family	9.4	10.3	38
Bipolar I/schizoaffective	23	13	12
Bipolar II	10	0	0
Recurrent major depression	13	10	1
Single-episode major depression	11	0	2
Other psychiatric diagnosis	19	6	3
Average affecteds per family[a]	3.5	7.7	13
Unilineal families[b]	10	2	0
Parent of origin (paternal/maternal/other)[c]	2/5/6	0/1/2	0/0/1

[a]Affecteds per family is based on a definition of affected which includes Bipolar I, Bipolar II, schizoaffective and recurrent major depression.
[b]Families are broadly defined as unilineal if at no mating do both parents have diagnoses of bipolar, schizoaffective or recurrent depression disorders.
[c]Families in which both maternal and paternal transmission is present or in which the parent of origin cannot be established are classified as other.

extensive training in the use of the instrument. Diagnoses were determined by review of the SCID interview, information from at least one family informant, and medical records, by a committee of psychiatric clinicians who were blind to both genotypes and family membership. Diagnostic reliability was monitored both within-site and between sites by annual blind review of videotaped SCID interviews, and was consistently high. An exception is family 16, which was ascertained from the NIMH intramural program in Bethesda. In this case, diagnoses were made in similar fashion, but interviews were conducted by two of us (J.R.K. and M.H.R.) using the SADS-L [Endicott and Spitzer, 1978] modified for DSM-III-R diagnoses.

A portion of Old Order Amish pedigree 110 was also included in this study. The advantages of the Old Order Amish for study, and the methods used in ascertainment and diagnosis, have been reviewed elsewhere [Hostetter et al., 1983; Egeland et al., 1990]. Briefly, subjects were interviewed using the SADS-L, and research diagnostic criteria (RDC) [Spitzer et al., 1978] diagnoses were made by a panel of psychiatric clinicians blind to genotype or family membership. Information from the interview, from at least one family informant, and from medical records was incorporated in this process. A portion of pedigree 110 was selected as more likely to be unilineal in transmission of affective disorder and was included in this study.

Three families from New York were selected for high density of affective illness from Albert Einstein College of Medicine hospitals and clinics. All family members were interviewed using a version of the SADS-L modified in order to obtain additional information regarding bipolar disorders (SADS-LB). The interview data and medical records were reviewed by a panel of two clinical research psychiatrists and a senior research social worker who adhered to RDC criteria for developing consensus diagnoses. This diagnostic panel was blind to genotypic information.

The UCSD and UBC families derive from similar general North American populations, and were obtained through carefully coordinated ascertainment and diagnostic procedures. For these reasons, these families have been treated as one population in our analyses. The three resulting populations (UCSD/UBC, Amish, and New York) are compared in terms of a variety of clinical characteristics in Table I. They differ primarily in that the Amish family is larger, and that the Amish and New York families have more affecteds per family. The UCSD/UBC families have more subjects with bipolar II disorder. The UCSD/UBC and Amish families also have a greater ratio of bipolar to unipolar diagnoses than do the New York families.

Genotyping

The region of chromosome 22 surrounding the VCFS deletion site was examined using seven CA repeat microsatellite markers, which have been described in several recent maps of chromosome 22 [Gyapay et al., 1994; Murray et al., 1994; Morrow et al., 1995]. Their order, approximate map distances, and relationship to the VCFS region are illustrated in Figure 1. Two of these markers, D22S427 and D22S264 (heterozygosities 0.63 and 0.80), flank an approximately 2-cM region of chromosome 22q11 that contains the VCFS deleted region [Morrow et al., 1995]. The other five markers, D22S420, D22S306, D22S303, D22S315, and D22S311 (heterozygosities 0.77, 0.52, 0.65, 0.82, and 0.92, respectively), span an approximately 18-cM region flanking these two markers. In an initial screen, three of these markers (D22S427, D22S264, and D22S303) were examined in all 17 families. Subsequently, the other four markers were genotyped in selected families in order to confirm positive results from the first screen, or to obtain more linkage information from poorly informative families.

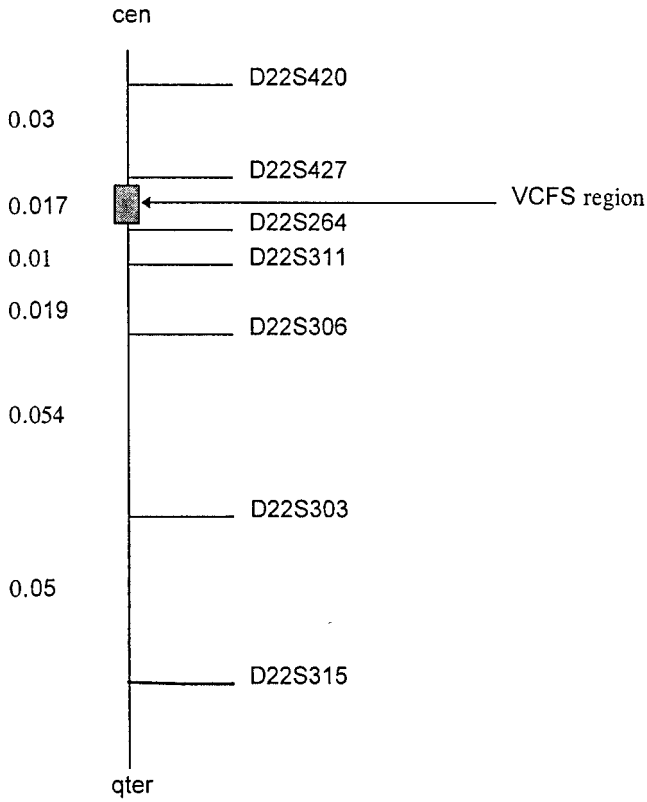

Fig. 1. Map of VCFS region on chromosome 22. Marker order and position of VCFS deletion region are derived from physical mapping data from overlapping yeast artificial chromosomes (YACs) [Morrow et al., 1995]. Recombination fractions, indicated at left, derive from an integration of several linkage maps [Gyapay et al., 1994; Murray et al., 1994], and must be considered an approximation.

High molecular weight DNA was extracted from peripheral blood leukocytes using standard techniques. Primer pairs were obtained from Research Genetics (Huntsville, AL). The forward primers were end-labeled using polynucleotide kinase (Boehringer-Mannheim, Indianapolis, IN) and ^{32}P-ATP. A polymerase chain reaction (PCR) was carried out in 20 μl using 25–50 ng of genomic DNA, 10 pmoles of labeled forward primer, 10 pmoles of unlabelled reverse primer, 1 unit of *taq* polymerase (Boehringer-Mannheim), 200 mM of each nucleotide, and buffer supplied by manufacturer. Cycling conditions included an initial 2-min denaturation step at 94°C followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 30 sec, with a final

3-min extension. Denaturing buffer (12.5 μl) containing 95% formamide, 10 mM EDTA, pH 8.0, and 0.1% each of bromophenol blue and xylene cyanol was added to the reaction and, following heat denaturation, a 2–3-μl aliquot was loaded onto either a 5% or 6% acrylamide gel containing 7 M urea. To ensure quality control, a set of common DNA samples was used for each gel. In addition, several samples were included from gels that had been previously analyzed. The samples were electrophoresed at 45 watts for 2–2.5 hr. Following electrophoresis, the gels were dried and exposed to X-ray film for a period of 2–16 hr. Autoradiograms were analyzed blind to diagnosis.

Linkage Analyses

The power of the pedigree collection was estimated using the program SIMLINK [Ploughman and Boehnke, 1989]. Pedigree data were simulated under an assumption of linkage between the disease and a biallelic marker whose true recombination fraction was either 0.0 or 0.05. Fifty percent heterogeneity was also assumed in the simulation. The simulated data were then analyzed under an assumption of heterogeneity at a variety of recombination fractions in order to obtain the estimated mean maximum lod score.

Parametric linkage analyses were conducted using the MLINK program from the LINKAGE 5.1 program package [Lathrop et al., 1984]. Analyses employed a definition of "affected" that included bipolar I, bipolar II, schizoaffective, and recurrent major depression diagnoses. One subject had a diagnosis of schizophrenia and was considered to have an unknown affection status. Subjects with other psychiatric diagnoses were also considered unknown. Three models of genetic transmission were tested as shown in Table II. In the table, model 1 is a high-penetrance dominant model derived from a segregation analysis in the Old Order Amish, and was used in previous studies of this population [Egeland et al., 1987; Kelsoe et al., 1989]. Models 2 and 3 are moderate-penetrance dominant and recessive models, respectively. Age-dependent penetrance was modeled using five liability classes which increased linearly from age 14 to a maximum at age 30. Allele frequencies were obtained from the Genome Database (Johns Hopkins University) or from published reports of these markers [Gyapay et al., 1994; Buetow et al., 1993], except for D22S427, where they were estimated from 66 control chromosomes, and D22S311, where allele frequencies were assumed equal. The HOMOG program was used for analyses under an assumption of heterogeneity [Ott, 1991].

Nonparametric analyses were performed using an identity-by-state approach, the affected pedigree member method (APM) [Weeks and Lange, 1988], and an identity-by-descent approach, i.e., the affected sib-pair method. Both analyses employed the same diagnostic model as the lod score analyses. For the APM analyses, the f(p) = 1/SQR weighting function for allele frequency was used because it has been shown to have the greatest statistical power, and it is recommended by the designers of the test. Empirical *P* values were estimated by simulation using the SIM, SIMMULT, and HIST programs from the APM package. A multipoint APM analysis incorporated the three markers D22S427, D22S264, and D22S303, using map distances as indicated in Figure 1. Affected sib-pair analyses were conducted using the SIBPAL program [S.A.G.E., 1994]. In these analyses, only results from affected sib pairs were considered, and sample size was corrected for nonindependence of multiple pairs derived from the same sibship.

Pedigrees and genotypic data will be provided to interested investigators upon request.

TABLE II. Models for Linkage Analysis*

Model	q	Mode	f1	f2	f3
1	0.021	Dominant	0.85	0.85	0.001
2	0.024	Dominant	0.5	0.5	0.001
3	0.218	Recessive	0.5	0.001	0.001

*q = frequency of disease gene; f1 = penetrance of AA, where A is the disease allel; f2 = penetrance of Aa; f3 = penetrance of aa.

RESULTS

Power analyses of the family collections were first performed by simulation in order to determine the approximate lod scores likely to be obtained if a marker was linked to bipolar disorder. The results of these simulation studies are summarized in Table III for the combined families, and for the UCSD/UBC families where the highest actual lod scores were obtained. Only under the recessive model at θ = 0.0 was the estimated maximum lod >3.0 for the combined families. Under the dominant models, estimated mean maximum lods under linkage and 50% heterogeneity ranged from 1.3–2.05 for the combined families, and from 0.64–1.15 for the UCSD/UBC families.

Table IV summarizes the lod scores for each population and for the combined family set for the three markers used in the initial screen. A maximum lod score of 2.51 was obtained at D22S303 in the UCSD/UBC families under model 2. Lod scores of 1.87 and 0.70 were also obtained at this marker in this population under models 1 and 3, respectively. In the Amish family, a maximum lod of 0.69 was obtained at D22S303 under model 3. At D22S303 in the combined families, lod scores of 1.68 and 1.28 were obtained under models 2 and 3, respectively. For both the UCSD/UBC families and the combined families, these lod scores are comparable to or exceed those expected from the simulation studies under model 2. The maximum lod score for D22S427 was 0.72 in the UCSD/UBC families under

TABLE III. Statistical Power of the Family Collection

		Estimated mean maximum lod score under linkage	
Population	Model	θ = 0.0	θ = 0.05
UCSD/UBC	1	1.152	0.862
	2	0.756	0.639
	3	1.642	1.251
Combined	1	2.053	1.542
	2	1.639	1.319
	3	3.356	2.267

TABLE IV. Lod Scores for Chromosome 22 Markers*

Locus	Population	Model	Theta 0.00	0.01	0.05	0.10	0.20	0.30	0.40
D22S427	UCSD/UBC	1	−1.10	−0.90	−0.36	0.05	0.39	0.38	0.20
		2	0.22	0.33	0.58	**0.72**	0.71	0.50	0.22
		3	−2.23	−2.00	−1.32	−0.78	−0.24	−0.04	0.00
	Amish	1	−3.50	−2.62	−1.60	−1.02	−0.46	−0.21	−0.08
		2	−1.95	−1.59	−1.00	−0.63	−0.26	−0.10	−0.02
		3	−0.19	−0.16	−0.07	0.00	0.04	0.03	0.01
	New York	1	−1.18	−1.13	−1.00	−0.91	−0.68	−0.39	−0.16
		2	−0.98	−0.97	−0.92	−0.83	−0.57	−0.31	−0.12
		3	−1.58	−1.39	−0.94	−0.62	−0.29	−0.13	−0.05
	Total	1	−5.78	−4.65	−2.97	−1.89	−0.75	−0.22	−0.03
		2	−2.71	−2.24	−1.34	−0.75	−0.12	0.10	0.08
		3	−3.99	−3.55	−2.32	−1.40	−0.48	−0.15	−0.04
D22S264	UCSD/UBC	1	−5.33	−4.34	−2.45	−1.21	−0.08	0.24	0.17
		2	−2.44	−2.07	−1.10	−0.38	0.26	0.34	0.18
		3	−3.69	−3.33	−2.29	−1.45	−0.55	−0.18	−0.06
	Amish	1	−2.78	−1.90	−1.42	−1.33	−1.09	−0.64	−0.26
		2	−1.56	−1.19	−0.80	−0.68	−0.54	−0.33	−0.14
		3	−0.19	−0.16	−0.07	0.00	0.05	0.04	0.03
	New York	1	−1.30	−1.15	−0.87	−0.71	−0.48	−0.27	−0.11
		2	−1.11	−1.01	−0.78	−0.61	−0.36	−0.19	−0.07
		3	−1.58	−1.42	−0.99	−0.67	−0.32	−0.15	−0.06
	Total	1	−9.41	−7.39	−4.74	−3.25	−1.65	−0.67	−0.20
		2	−5.11	−4.27	−2.68	−1.67	−0.64	−0.18	−0.03
		3	−5.45	−4.91	−3.34	−2.12	−0.82	−0.29	−0.09
D22S303	UCSD/UBC	1	1.63	1.73	**1.87**	1.81	1.40	0.85	0.33
		2	**2.51**	2.46	2.24	1.96	1.36	0.78	0.29
		3	0.22	0.34	0.61	**0.70**	0.57	0.31	0.08
	Amish	1	−0.60	−0.64	−0.77	−0.85	−0.69	−0.43	−0.21
		2	−0.03	−0.05	−0.16	−0.27	−0.34	−0.27	−0.15
		3	**0.69**	0.67	0.59	0.49	0.31	0.17	0.06
	New York	1	−0.88	−0.87	−0.76	−0.58	−0.29	−0.11	−0.02
		2	−0.81	−0.79	−0.70	−0.54	−0.28	−0.11	−0.03
		3	0.10	0.09	0.08	0.07	0.04	0.02	0.01
	Total	1	0.15	0.22	0.34	0.38	0.43	0.31	0.10
		2	**1.68**	1.61	1.38	1.15	0.75	0.40	0.11
		3	1.00	1.10	**1.28**	1.26	0.92	0.49	0.15

*Maximum lod scores >0.50 are in boldface.

model 2. No notable positive lods were obtained at D22S264 in any of the populations, nor in the New York families at any of the three markers. Analyses under heterogeneity using HOMOG yielded results similar to those under homogeneity. Under model 2 at D22S303, maximum lod scores under heterogeneity were 2.51 in the UCSD/UBC families, and 1.71 in the combined families. No significant evidence of heterogeneity was detected.

Examination of lods in individual families indicates that positive lods are found in multiple families and at multiple markers. This argues against the results being an isolated chance positive, and lends further support to the possibility of real linkage. As seen in Table V, positive lods at D22S303 are found primarily in the UCSD/UBC families 24, and 16, and in Amish pedigree 110 (Family 5001). The other two markers included in the primary screen, D22S427 and D22S264, also yielded slightly positive lods ranging from 0.37–0.57 in the three UCSD/UBC families 22, 24, and 72. Families with positive lods were examined in more detail using the four markers in the secondary screen. Positive lods were detected with three of these markers in the three UCSD/UBC families 16, 22 and 24. The highest individual lods obtained were 1.10 at D22S420 in family 16, and 1.07 at D22S303 in family 24. The expected lod scores for each of the family-model combinations as determined in the simulation studies are also presented in Table V. In each case, except for the Amish family, these positive lod scores exceed those expected under linkage. It is of interest that positive lods were ob-

TABLE V. Positive Lod Scores in Individual Families

Locus	Family[a]	Model	Theta	lod	Expected lod (simulated)[b]
D22S303	24	1	0	1.07	0.18
D22S303	5001	3	0	0.69	1.45
D22S303	16	2	0	0.60	0.36
D22S264	24	2	0	0.57	0.18
D22S264	72	2	0	0.46	0.14
D22S427	22	2	0	0.37	0.15
D22S420	16	2	0	1.10	0.36
D22S420	22	2	0	0.37	0.15
D22S306	22	1	0	0.82	0.16
D22S306	22	3	0	0.70	0.47
D22S306	22	2	0	0.68	0.15
D22S315	24	3	0	0.42	0.26

[a]Families 16, 22, and 24 are from the UCSD/UBC set; family 72 is from the New York set; and family 5001 is Old Order Amish pedigree 110.
[b]Estimated mean maximum lod score for the given model and recombination distance, as determined by simulation using SIMLINK.

tained under both dominant and recessive models for different family-marker combinations. This would be consistent with allelic heterogeneity at a disease locus. Overall, positive lods were obtained with markers spanning the entire 18-cM interval examined, and the two lods over 1.0 came from markers separated by 13 cM and flanking the VCFS region.

These results were further explored using the nonparametric affected pedigree member (APM) method, as shown in Table VI. The results were consistent with those obtained in the lod score analyses. In the combined families, only D22S303 yielded suggestive evidence of linkage in single-locus analyses ($P = 0.018$). A multipoint APM analysis incorporating all three markers in the primary screen yielded results similarly suggestive of linkage ($P = 0.017$). Stronger evidence was seen in the UCSD/UBC families alone, with suggestive evidence of linkage obtained at both D22S427 ($P = 0.031$) and D22S303 ($P = 0.003$). The multipoint analysis in this population yielded the strongest results for this test ($P = 0.0024$).

Similar results were obtained using the affected sib-pair method as summarized in Table VII. Results suggestive of linkage were obtained at D22S303 in the combined family set ($P = 0.004$), as well as in the UCSD/UBC families ($P = 0.017$) and the Amish family ($P = 0.044$) examined separately. No positive evidence was obtained at the other two markers from the initial screen.

Since this region of chromosome 22 has also been implicated in linkage studies of schizophrenia, we considered the hypothesis that the disease susceptibility locus might predispose more generally to psychosis, rather than specifically to bipolar disorder. To test this hypothesis, the lod score analyses were repeated, considering as affected only those subjects who manifested marked psychotic symptoms. Only three families were informative for linkage under this definition of affection status (families 10, 72, and 230). The maximum lod score obtained was 0.46 for family 72, at D22S264 under model 2. Therefore, only very limited support for this hypothesis could be obtained. Rather, the suggestive evidence for linkage to bipolar disorder comes almost exclusively from nonpsychotic subjects.

TABLE VI. Affected Pedigree Member Analyses

Population	Locus	Statistic[a]	Empirical P value[b]
Combined	D22S427	1.32	0.133
	D22S264	0.81	0.183
	D22S303	2.32	0.018
	Multipoint[c]	2.41	0.017
UCSD/UBC	D22S427	2.02	0.031
	D22S264	1.27	0.1
	D22S303	3.15	0.003
	Multipoint[c]	3.30	0.0024
Amish			Not significant
New York			Not significant

[a]These APM statistics were calculated using the 1/SQR function for weighting of allele frequency, which yields an intermediate weighting factor and is recommended by the authors of the analysis program.
[b]Empirical P values were calculated by simulation using 1,000 replicates and the programs SIM, SIMMULT, and HIST.
[c]Multipoint APM incorporates the above three markers and map distances, as indicated in Figure 1.

TABLE VII. Affected Sib-Pair Analyses at D22S303

Population	No. pairs	Proportion of alleles i.b.d.	P
UCSD/UBC	21	0.610	0.017
Amish	7	0.679	0.044
New York	Not significant		
Combined	29	0.619	0.004

DISCUSSION

These data are consistent with the hypothesis that a bipolar susceptibility gene may exist on chromosome 22q11 in a subset of affected families. However, none of these results reach the conventional level for statistical significance of a lod score of 3.0 or 1,000:1 odds in favor of linkage. For this reason, conclusions regarding these data must be qualified as only suggestive of linkage. The finding of positive lods in multiple families at multiple markers in this region does, to some extent, argue against this being an isolated false-positive. Furthermore, some have argued that in examining a candidate gene with a specific a priori hypothesis, a less stringent lod score criterion is indicated. Ott, [1991] however, argues that given the history of candidate gene studies, and the fact that they are usually conducted as part of a genome survey, the conventional level of 3.0 should be retained. In either case, given the complexities and uncertainties of the mode of transmission of bipolar disorder, the meaning of linkage results of this magnitude is unclear.

Nevertheless, these results are interesting and warrant further investigation in other collections of bipolar families. Published reports to date in which chromosome 22q markers have been examined in bipolar families indicate no evidence for linkage [Coon et al., 1993; Detera-Wadleigh et al., 1994]. However, the markers used in these studies did not adequately evaluate the VCFS deleted region. Further studies of bipolar families using microsatellite markers in this region are required. These results are also intriguing in the light of recent reports of possible linkage of schizophrenia to markers at 22q11.2–22q13 [Pulver et al., 1994b; Lasseter et al., 1995]. Though these results have not been uniformly replicated, the possibility of a common susceptibility locus for psychosis should not be completely excluded [Pulver et al., 1994a; Polymeropoulos et al., 1994; Coon et al., 1994].

Although this region of chromosome 22 was analyzed in the context of VCFS, the marker that consistently yielded the maximal lod scores was D22S303, which is approximately 5 cM telomeric to the VCFS deleted region. In general, negative lod scores were obtained for markers D22S427 and D22S264, which flank the commonly deleted region. This could suggest that chromosome 22q11 has two bipolar susceptibility loci, one in the VCFS deleted region, which is involved in the psychiatric manifestations of VCFS, and another near D22S303. Consistent with this view are recent findings regarding one candidate gene for the psychiatric manifestations of VCFS, catechol-O-methyltransferase (COMT). COMT is one of the major enzymes responsible for catecholamine inactivation [Axelrod and Tom-

chick, 1958], and is deleted in most, if not all, patients with VCFS [Winqvist et al., 1992; Grossman et al., 1992; Scambler et al., 1992]. We recently characterized a low-activity variant of COMT that is associated with bipolar spectrum disorders occurring in VCFS, especially rapid-cycling bipolar disorder [Lachman et al., 1996a,b]. However, recent findings from our laboratory suggest that the low-activity variant does not exert a major gene effect in bipolar disorder occurring in the general population [Lachman et al., 1997]. One interesting candidate gene that maps close to D22S303 is the G-protein alpha subunit, G_z, which is a member of a gene family considered to be a feasible candidate for bipolar disorder [Lachman and Papolos, 1989; Schreiber and Avissar, 1991; Blatt et al., 1988].

In summary, we examined the VCFS region in 17 bipolar families using seven microsatellite markers, and found results suggestive, but not conclusive, of linkage. In a genetically heterogeneous condition, confirmation of linkage and the extraction of informative mapping data may require an analysis of hundreds of families.

ACKNOWLEDGMENTS

H.M.L. is supported by the Carmel Hill Fund and is a recipient of the Stanley Foundation Research Award from the National Alliance for the Mentally Ill. D.F.P. is a recipient of an NIMH Physician Scientist Career Development Award. This work has also been supported in part by the Department of Veterans Affairs, the NIMH (grant MH47612), the UCSD Mental Health Clinical Research Center (grant MH30914), and the MacArthur Foundation (UCSD and UBC). Portions of this work were also supported by the UCSD General Clinical Research Center (grant M01 RR00827). Some of the analyses were performed using the program package S.A.G.E. (grant 1 P41 RR03655). The authors thank Drs. Raju Kucherlapati and Bernice Morrow for mapping data from the VCFS region and for access to unpublished markers in this region. We also acknowledge the generous assistance of Dr. Janice Egeland in providing clinical information regarding the Amish sample, and assistance in the diagnostic assessment of one of the New York families. Jean Endicott and Johnine Cummings also provided assistance in assessing one of the New York families. We thank Dr. J. Christian Gillin for the support of the UCSD Mental Health Clinical Research Center, and Drs. Maureen Morison and Arvin Mirow for assistance with ascertainment and diagnosis of the UCSD families.

REFERENCES

Axelrod J, Tomchick R (1958): Enzymatic O-methylation of epinephrine and other catechols. J Biol Chem 233:702–705.

Baron M, Risch N, Hamburger R, Mandel B, Kushner S, Newman M, Drumer D, Belmaker RH (1987): Genetic linkage between X-chromosome markers and bipolar affective illness. Nature 326:289–292.

Baron M, Freimer NB, Risch N, Lerer B, Alexander JR, Straub RE, Asokan S, Das K, Peterson A, Amos J, Endicott J, Ott J, Gilliam TC (1993): Diminished support for linkage between manic depressive illness and X-chromosome markers in three Israeli pedigrees. Nat Genet 3:49–55.

Berrettini WH, Goldin LR, Gelernter J, Gejman PV, Gershon ES, Detera-Wadleigh S (1990): X-chromosome markers and manic-depressive illness. Rejection of linkage to Xq28 in nine bipolar pedigrees. Arch Gen Psychiatry 47:366–373.

Berrettini WH, Ferraro TN, Goldin LR, Weeks DE, Detera-Wadleigh S, Nurnberger JI, Gershon ES (1994): Chromosome 18 DNA markers and manic-depressive illness: Evidence for a susceptibility gene. Proc Natl Acad Sci USA 91:5918–5921.

Bertelsen A, Harvald B, Hauge M (1977): A Danish twin study of manic-depressive disorders. Br J Psychiatry 130:330–351.

Blackwelder WC, Elston RC (1985): A comparison of sib-pair linkage tests for disease susceptibility loci. Genet Epidemiol 2:85–97.

Blackwood DHR, He L, Morris SW, McLean A, Whitton C, Thomson M, Walker MT, Woodburn K, Sharp CM, Wright AF, Shibasaki Y, St. Clair DM, Porteous DJ, Muir WJ (1996): A locus for bipolar affective disorder on chromosome 4p. Nat Genet 12:427–430.

Blatt C, Eversole-Cire P, Cohn VH, et al. (1988): Chromosome localization of genes encoding guanine nucleotide-binding protein subunits in mouse and human. Proc Natl Acad Sci USA 85:7642–7646.

Buetow KH, Duggan D, Yang B, Ludwigsen S, Puck J, Porter J, Budarf M, Spielman R, Emanuel BS (1993): A microsatellite-based multipoint index map of human chromosome 22. Genomics 18:329–339.

Chow EWC, Bassett AS, Weksberg R (1994): Velo-cardio-facial syndrome and psychotic disorders: Implications for psychiatric genetics. Am J Med Genet 54:107–112.

Coon H, Jensen S, Hoff M, Holik J, Plaetke R, Reimherr F, Wender P, Leppert M, Byerley W (1993): A genome-wide search for genes predisposing to manic-depression, assuming autosomal dominant inheritance. Am J Hum Genet 52:1234–1249.

Coon H, Holik J, Hoff M, Reimherr F, Wender P, Myles-Worsley M, Waldo M, Freedman R, Byerley W (1994): Analysis of chromosome 22 markers in nine schizophrenia pedigrees. Am J Med Genet 54:72–79.

De Bruyn A, Mendelbaum K, Sandkuijl LA, Delvenne V, Hirsch D, Staner L, Mendlewicz J, Van Broeckhoven C (1994): Nonlinkage of bipolar illness to tyrosine hydroxylase, tyrosinase, and D2 and D4 dopamine receptor genes on chromosome 11. Am J Psychiatry 151:102–106.

Detera-Wadleigh SD, Hsieh WT, Berrettini WH, Goldin LR, Rollins DY, Muniec D, Grewal R, Guroff JJ, Turner G, Coffman D, Barrick J, Mills K, Murray J, Donohue SJ, Klein DC, Sanders J, Nurnberger JI, Gershon ES (1994): Genetic linkage mapping for a susceptibility locus to bipolar illness—Chromosomes 2, 3, 4, 7, 9, 10p, 11p, 22, and Xpter. Am J Med Genet 54:206–218.

Egeland JA, Gerhard DS, Pauls DL, Sussex JN, Kidd KK, Allen CR, Hostetter AM, Housman DE (1987): Bipolar affective disorders linked to DNA markers on chromosome 11. Nature 325:783–787.

Egeland JA, Sussex JN, Endicott J, Hostetter AM, Offord DR, Schwab JJ, Allen CR, Pauls DL (1990): The impact of diagnoses on genetic linkage study for bipolar affective disorders among the amish. Psychiatr Genet 1:5–18.

Endicott J, Spitzer R (1978): A diagnostic interview: The Schedule for Affective Disorders and Schizophrenia. Arch Gen Psychiatry 35:837–844.

Faraone SV, Kremen WS, Tsuang MT (1990): Genetic transmission of major affective disorders: Quantitative models and linkage analyses. Psychol Bull 108:109–127.

Freimer NR, Reus VI, Escamilla MA, et al. (1996): Genetic mapping using haplotype, association and linkage methods suggests a locus for severe bipolar disorder (BPI) at 18q22–q23. Nat Genet 12:436–441.

Gershon ES (1991): Marker genotyping errors in old data on X-linkage in bipolar illness. Biol Psychiatry 29:721–729.

Ginns El, Ott J, Egeland JA, et al. (1996): A genome-wide search for chromosomal loci linked to bipolar disorder in the Old Order Amish. Nat Genet 12:431–435.

Goldberg R, Motzkin B, Marion R, Scrambler PJ, Shprintzen RJ (1993): Velo-cardio-facial syndrome, a review of 120 patients. Am J Med Genet 45:313–319.

Grossman MH, Emanual BS, Budarf ML (1992): Chromosomal mapping of the human catechol-O-methyl transferase gene to 22q11.1–q11.2. Genomics 2:822–825.

Gurling H, Smyth C, Kalsi G, Moloney E, Rifkin L, O'Neill J, Murphy P, Curtis D, Petursson H, Brynjolfsson J (1995): Linkage findings in bipolar disorder. Nat Genet 10:8–9.

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994): The 1993–94 Genethon human genetic linkage map. Nat Genet 7:246–339.

Holmes D, Brynjolfsson J, Brett P, Curtis D, Petursson H, Sherrington R, Gurling H (1991): No evidence for a susceptibility locus predisposing to manic depression in the region of the dopamine (D2) receptor gene. Br J Psychiatry 158:635–641.

Hostetter AM, Egeland JA, Endicott J (1983): Amish Study, II: Consensus diagnoses and reliability results. Am J Psychiatry 140:62–66.

Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, Long RT, Kidd KK, Conte G, Housman DE, Paul SM (1989): Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. Nature 342:238–243.

Lachman HM, Papolos D (1989): Abnormal signal transduction: A hypothetical model for bipolar affective disorder. Life Sci 45:1413–1426.

Lachman H, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996a): Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250.

Lachman HL, Morrow B, Shprintzen R, Veit S, Parsia S, Faedda G, Goldberg R, Kucherlapati R, Papolos D (1996b): Association of codon 108/158 catechol-O-methyltransferase gene polymorphism with the psychiatric manifestations of velo-cardio-facial-syndrome. Am J Med Genet 67:468–472.

Lachman HM, Kelsoe JR, Moreno L, Katz S, Papolos DF (1997): Lack of association of catechol o-methyltransferase (COMT) functional polymorphism in bipolar affective disorder. Psychiatric Genet (in press).

Lasseter VK, Pulver AE, Wolyniec PS, Nestadt G, Meyers D, Karayiorgou M, Housman D, Antonarakis S, Kazazian H, Kasch L (1995): Follow-up report of potential linkage for schizophrenia on chromosome 22q: Part 3. Am J Med Genet 60:172–173.

Lathrop GM, Lalouel JM, Julier C, Ott J (1984): Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446.

Lindsay EA, Halford S, Wadey R, Scambler PJ, Baldini A (1993): Molecular and cytogenetic characterization of the DiGeorge syndrome regions using fluorescence in situ hybridization. Genomics 17:403–407.

Lindsay EA, Morris MA, Gos A, Nestadt G, Wolyniec PS, Lasseter VK, Shprintzen R, Antonarakis SE, Baldini A, Pulver AE (1995): Schizophrenia and chromosomal deletions within 22q11.2. Am J Hum Genet 56:1502–1503.

Mitchell P, Selbie L, Waters B, Donald J, Vivero C, Tully M, Shine J (1992): Exclusion of close linkage of bipolar disorder to dopamine D1 and D2 receptor gene markers. J Affective Disord 25:1–11.

Morrow B, Goldberg R, Carlson C, Gupta RD, Sirotkin H, Collins J, Dunham I, O'Donnell H, Scambler PJ, Shprintzen R, Kucherlapati R (1995): Molecular definition of the 22q11 deletions in velo-cardio-facial syndrome. Am J Hum Genet 56:1391–1403.

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, Sheffield VC, Sunden S, Duyk GM (1994): A comprehensive human linkage map with centimorgan density. Cooperative Human Linkage Center (CHLC). Science 265:2049–2054.

Nurnberger JI, Gershon ES (1984): Genetics of affective disorders. In Post RM, Ballanger JC (eds): "Neurobiology of Mood Disorders." Baltimore: William and Wilkins, pp 76–101.

Nurnberger JI, Goldin LR, Gershon ES (1994): Genetics of Psychiatric Disorders In Winokur G, Clayton P (eds): "The Medical Basis of Psychiatry." New York: W.B. Saunders, pp 459–492.

Ott J (1991): "Analysis of Human Genetic Linkage." Baltimore: Johns Hopkins University Press, p 247.

Papolos DF, Faedda GL, Veit S, Goldberg R, Morrow B, Kucherlapati R, Shprintzen RJ (1996): Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial-syndrome: does hemizygous deletion of chromosome 22q11 result in bipolar affective disorder. Am J Psychiatry 153:1541–1547.

Ploughman LM, Boehnke M (1989): Estimating the power of a proposed linkage study for a complex genetic trait. Am J Hum Genet 44:543–551.

Polymeropoulos MH, Coon H, Byerley W, Gershon ES, Goldin L, Crow TJ, Rubenstein J, Hoff M, Holik J, Smith Am (1994): Search for a schizophrenia susceptibility locus on human chromosome 22. Am J Med Genet 54:93–99.

Pulver AE, Karayiorgou M, Lasseter VK, Wolyniec P, Kasch L, Antonarakis S, Housman D, Kazazian HH, Meyers D, Nestadt G (1994a): Follow-up of a report of a potential linkage for schizophrenia on chromosome 22q12–q13.1: Part 2. Am J Med Genet 54:44–50.

Pulver AE, Karayiorgou M, Wolyniec PS, Lasseter VK, Kasch L, Nestadt G, Antonarakis S, Housman D, Kazazian HH, Meyers D (1994b): Sequential strategy to identify a susceptibility gene for schizophrenia: Report of potential linkage on chromosome 22q12–q13.1: Part 1. Am J Med Genet 54:36–43.

Pulver AE, Nestadt G, Goldberg R, Shprintzen RJ, Lamacz M, Wolyniec PS, Morrow B, Karayiorgou M, Antonarakis SE, Housman D (1994c): Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. J Nerv Ment Dis 182:476–478.

Rice J, Reich T, Andreasen NC, Endicott J, van Eerdewegh M, Fishman R, Hirschfeld RM, Klerman GL (1987): The familial transmission of bipolar illness. Arch Gen Psychiatry 44:441–47.

Risch N (1990): Linkage strategies for genetically complex traits. II. The power of affected relative pairs. Am J Hum Genet 46:229–241.

Sadovnick AD, Remick RA, Lam R, Zis AP, Yee IM, Huggins MJ, Baird PA (1994): A mood disorder service genetic database: Morbidity risks for mood disorders in 3,942 first-degree relatives of 671 index cases with single depression, recurrent depression, bipolar I or bipolar II. Am J Med Genet 54:132–140.

S.A.G.E (1994): Statistical Analysis for Genetic Epidemiology. Version 2.6. Computer program package available from the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Scambler PJ, Kelly D, Lindsay E, Williamson R, Goldberg R, Shprintzen R, Wilson DI, Goodship JA, Cross IE, Burn J (1992): Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. Lancet 339:1138–1139.

Schreiber G, Avissar S (1991): Lithium sensitive G protein hyperfunction: A dynamic model for the pathogenesis of bipolar affective disorder. Med Hypotheses 35:237–243.

Sham PC, Morton NE, Rice JP (1992): Segregation analysis of the NIMH collaborative study: Family data on bipolar disorder. Psychiatr Genet 2:175–184.

Shprintzen RJ, Goldberg RB, Lewin ML, Sidoti EJ, Berkman MD, Argamaso RV, Young D (1978): A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: Velo-cardio-facial syndrome. Cleft Palate J 15:56–62.

Shprintzen RJ, Goldberg R, Golding-Kushner KJ, Marion RW (1992): Late-onset psychosis in the velo-cardio-facial syndrome. Am J Med Genet 42:141–142.

Spence MA, Flodman PL, Sadovnick AD, Bailey-Wilson JE, Ameli H, Remick RA (1995): Bipolar disorder: Evidence for a major locus. Am J Med Genet 60:370–376.

Spitzer R, Endicott J, Robins E (1978): Research diagnostic criteria: Rationale and reliability. Arch Gen Psychiatry 35:773–782.

Spitzer RL, Williams JBW, Gibbon M (1987): "Structured Clinical Interview for DSM-3-R (SCID)." New York: New York State Psychiatric Institute, Biometrics Research.

Stine OC, Xu J, Koskela R, McMahon FJ, Geschwend M, Friddle C, Clark CD, McInnis MG, Simpson SG, Breschel TS, Vishio E, Riskin K, Feilotter H, Chen E, Shen S, Folstein S, Meyers DA, Botstein D, Marr TG, DePaulo JR (1995): Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. Am J Hum Genet 57:1384–1394.

Straub RE, Lehner T, Luo Y, Loth JE, Shao W, Sharpe L, Alexander JR, Das K, Simon R, Fieve RR, Lerer B, Endicott J, Ott J, Gilliam TC, Baron M (1994): A possible vulnerability locus for bipolar affective disorder on chromosome 21q22.3. Nat Genet 8:291–296.

Weeks DE, Lange K (1988): The affected-pedigree-member method of linkage analysis. Am J Hum Genet 42:315–326.

Winqvist R, Lundstrom K, Salminen M, Laatikainen M, Ulmanen I (1992): The human catechol-O-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows a frequent RFLP with Bgl I. Cytogenet Cell Genet 59:253–257.